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Protentialites of Proteinoids for Nutritional Investigation

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RC#7

Comment on paper by Dr. Fox by Dr. A. T. McPherson, National Bureau of Standards:

Dr. McPherson: The pansynthesis of amino acids and the production of proteinoids represent a major breakthrough having consequences far beyond the relation to space flight possibilities. Heretofore, the production of a synthetic diet has involved the preparation of 18 to 22 individual amino acids; each by a long and laborious synthesis, and blending these in the optimum proportion, and the incorporation of these into polymers by yet to be developed methods, or the production of polypeptides by laborious synthesis.

Now, on the basis of work by Dr. Fox and others, the long and laborious methods that we foresaw have been short-circuited.

An analogous situation obtained in the early studies on synthetic rubber --- chemists in the 1920's thought that it might be necessary to build up polymers one unit at a time --- monomer to dimer to trimer, and so forth, adding each unit by a separate reaction. The actual synthesis turned out to be much simpler. All that was necessary was to emulsify the monomer with Ivory soap and water, add a little persulfate and a high molecular weight mercaptan. The polymer was formed quickly and engineering production became feasible.

It is interesting to speculate on the long range possibilities of synthetic food from the perspective of history. The discovery of agriculture 9000 years ago gave the basis for all that we call civilization. The world population 9000 years ago may have been one million with such keen competition for hunting and fishing rights that the earth was over-populated for the people then living. Today the population of the earth is 3 billion, an increase by a factor 3,000. Everyone is aware of the problem of population pressure. One possible effect of population pressure is accelerated research and development of synthetic food that may usher in a new era of civilization.

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Food may be regarded as predominantly a set of carbon compounds which have been selected by processes of Darwinian evolution. The ultimate method of preparation of such chemical substances may well be chemical synthesis. To deny this possibility is to ignore the accomplishments to date in the chemical synthesis of rubber, of polyamide fibers, of the hormones oxytocin and vasopressin, etc. In the field of food components, chemical synthesis already dominates the commerical production of vitamins. We already, therefore, are into the area of chemical synthesis of foods far more perhaps than is generally realized. This emphasis was made by McPherson in two papers in 1959 and 1961, the latter being titled "The Synthesis of Food" (1, 2).

Our own awareness of the potentiality in synthesis of food arose from our research on molecular evolution (3). The perspective which has been developed is covered in a paper from a symposium titled "Food of the Future" (3). Both Dr. McPherson and I use synthesis in the rigorous chemical sense, rather than in the sense of artificial assemblage of food components, synthetic or natural.

In order for chemical synthesis of food to be of interest socially it must, of course, be inexpensive. In order for the syntheses to be suitable for use on prolonged missions, such as Dr. Teller proposed for the surface of the moon, they must meet another prime requisite, that of simplicity of operation. The economical synthesis of vitamins referred to earlier, is in nearly every case extremely intricate organic chemistry and not at all suitable for execution, for example, in a space vehicle. The few amino acids which are used in supplementation can also be more economically synthesized than isolated. Their fabrication requires many steps of intricate organic chemistry; the application to space nutrition is hardly more thinkable than for the vitamins.

One can, however, now visualize simpler syntheses than the production, one at a time, of the many vitamins and amino acids, as these are carried out industrially. These food components are not only made one at a time

but the production of each amino acid or vitamin requires many steps and much processing.

The simultaneous synthesis, which we refer to as pansynthesis, of eight to eighteen amino acids, has been demonstrated to be a relatively simple process operationally. Mechanistically, from the viewpoint of the organic chemist, it must be very complicated, but operationally it is very simple. This kind of synthesis seemed more attainable following the finding that, under simple appropriate conditions, one could panpolymerize all of the eighteen amino acids common to protein to yield polyamino acids, proteinoids (5). These are nutritionally active (6).

This kind of investigation emerges from serious laboratory efforts to discipline theories of the origin of life (3). Such a background seems logical when we entertain two premises: (a) that the first organisms were chemically similar to contemporary cells and (b) that the first organisms would use, as food, substances chemically similar to those of which they were composed (7). The appropriate conditions are dry amino acids at the outset and a sufficient proportion of aneutral α-amino acids. The polymerization of non-α-amino acids is well known but this was not applicable to α-amino acids until the special conditions were discovered (5).

As a practical solution to the problem of space nutrition, the state of the art in synthesis of amino acids and proteinoids is not far advanced. We are not ready to substitute for a diet of roast chicken, potato salad, and apple strudel one of proteinoid, sugary condensation products of formaldehyde (4) and Hoffman La Roche vitamins. The possibilities of chemical, nutritional, and engineering investigations through the approach of pansynthesis are no longer, however, merely blackboard speculation.

I will report as concisely as possible upon salient features of the state of the art for proteinaceous compounds. Dr. Krampitz will, during the allotted time, present also some information on the nutritional availability to rats of the proteinoids.

Many syntheses of amino acids from simple gases are now known. The most recent of these and two of the earliest are shown in Table I. The two columns on the right indicate the production of four proteinaceous amino acids by electric discharge in gaseous mixture of methane, hydrogen, water and ammonia (8). As the columns on the left show, vapor phase thermal reactions of methane, ammonia, and water yield products readily hydrolyzable to amino acids (9). These synthesis have been carried out in beds of silicagel, silicasand, alumina, and volcanic beach sand. For three of these, the results are different; silica in either form gives similar results. Variation in the balance sheet with temperature is apparent. The first column indicates that amino acids are obtainable through synthesis carried out at 950° C in vapor phase reaction through silicasand. The second column also refers to experiments at 950° through silicagel; the first two columns show results which quantitatively are quite similar. The third column is at 1050° C. A greater variation in quantitative proportions is observable. The remarkable aspects of this pansynthesis are (a) that at least fourteen of the eighteen amino acids common to protein are synthesized by this process simultaneously and (b) all fourteen are amino acids common to protein. No others are found.

The four amino acids which are missing are tryptophan, histidine, cystine, and methionine. Tryptophan would have to be sought separately; such experiments have not been done. Histidine is blanketed on the amino acid analyzer by ammonia which is present in large proportions as one of the reactants. No cystine or methionine should be expected because of the fact that no sulphur compound has been used in the experiment reported here. Although basic amino acids are also formed, proportions have not yet been determined. The total yield is very low. It is not calculable for vapor phase reaction until further criteria are applied, but the unused gases could be

TABLE I

Amino Acid Compositions[a] Produced Thermally in the Presence of Silica and by Electric Discharge

	Thermal Synthesis			Electric Discharge Synthesis	
	Silicasand 950°C	Silicagel 950°C	Silicagel 1050°C	Spark Discharge[b]	Silent Discharge[b]
Aspartic acid	3.4%	2.5%	15.3%	0.3%	0.1%
Threonine	0.9	0.6	3.0	---	---
Serine	2.0	1.9	10.0	---	---
Glutamic acid	4.8	3.1	10.2	0.5	0.3
Proline	2.3	1.5	2.3	---	---
Glycine	60.3	68.8	24.4	50.8	41.4
Alanine	18.0	16.9	20.2	27.4	4.7
Valine	2.3	1.2	2.1	---	---
Alloisoleucine	0.3	0.3	1.4	---	---
Isoleucine	1.1	0.7	2.5	---	---
Leucine	2.4	1.5	4.6	---	---
Tyrosine	0.8	0.4	2.0	---	---
Phenylalanine	0.8	0.6	3.2	---	---
α-NH_2Butyric acid	0.8	---	---	4.0	0.6
β-Alanine	?[c]	?[c]	?[c]	12.1	2.3
Sarcosine	---	---	---	4.0	44.6
N-Methylalanine	---	---	---	0.8	6.5

[a] Basic amino acids were not listed in the table, because these amino acids were not yet fully studied. Some amino acid analyses of the thermal products showed peaks correspond with lysine (or ornithine) and arginine.

[b] Recalculated from the results obtained by Dr. S. L. Miller (8).

[c] β-Alanine peak obscured by other unknown peak.

Harada, K. and Fox, S. W. (1964) Nature 201, 335-336.

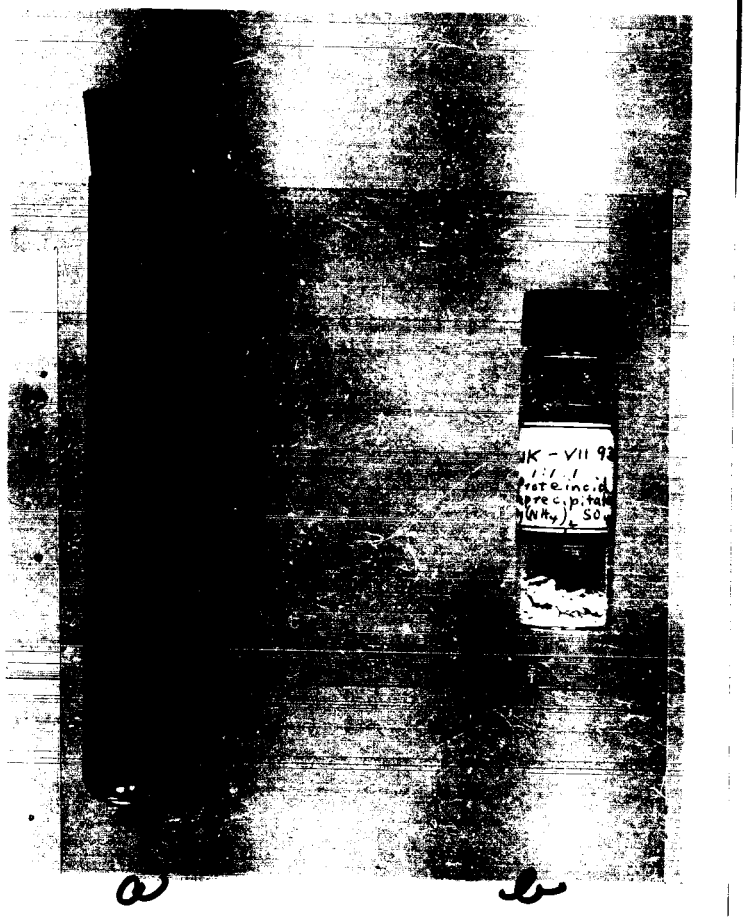


Fig. 1. a) Amino acids heated above the boiling point of water.
b) Ditto, with sufficient aspartic acid and glutamic acid, followed by purification by salting out.

recycled according to typical engineering concepts. Unwanted products could be converted to simple gases and run through a cycling process. The range of possibilities by varying solid support, temperature, etc., needs to be extensively investigated, but is obviously open to control.

Although I was originally asked only to discuss proteinoids, I have spent a few minutes with the synthesis of amino acids because this is a fundamental and prior problem in the context of some of the objectives of space nutrition.

Part of the background thinking about panpolymerization of amino acids is presented in Fig. 1. Many who are familiar with amino acids would expect by heating amino acids above the boiling point of water the dark, unworkable mass such as is seen in the tube on the left. Such a result, in fact, is well documented in the literature (10). If, however, one heats dry amino acids containing sufficient proportions of dicarboxylic amino acids at 170° for periods such as six hours, a light colored product results. It is soluble in dilute alkali and can be reprecipitated by salting out, a method classically used for the purification of proteins. The polymer contains some of each of the eighteen common amino acids. It has many of the properties of protein (3). Yields are typically 10-40%, higher figures being obtained by the addition of various phosphates. Copolymerization with aspartic acid can be employed with any number of amino acids from one to thirty. One can extend this operation to the nonproteinaceous amino acids. Extensive decomposition does not occur; in fact, 100% of amino acids can be recovered from moderately purified proteinoids (11). These reactions have now been repeated in many laboratories.

The degree of polymerization yields products in the range of molecular size of proteins (6). Proteinoids of mean molecular weight 3,500 - 8,500 are easily obtained. The synthesis and characterization of the proteinoids have been examined extensively (3, 6, 12).

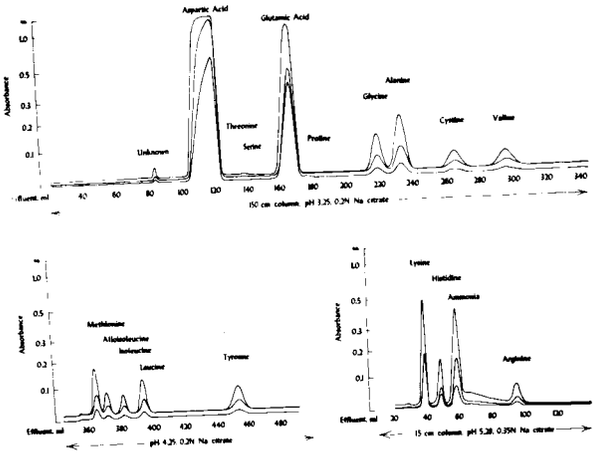
One salient structural feature is a very low degree of branching (13). Two properties of most pertinence here are (a) the proteinoids are split by proteolytic enzymes as studied in Dr. Krampitz's laboratory and in ours (6), and (b) nutritive quality.

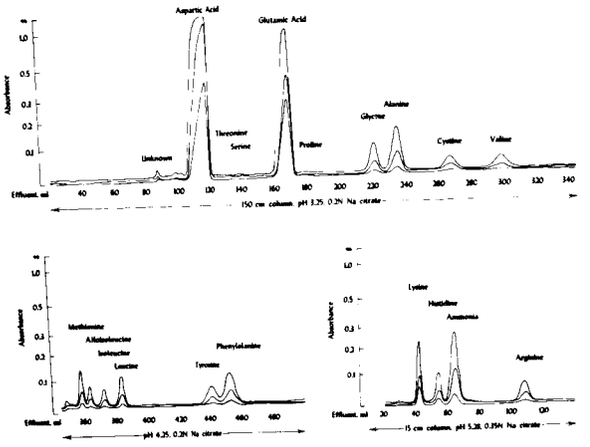
We first learned that proteinoids could be used instead of peptone by Lactobacillus arabinosus and by Proteus vulgaris. The former has amino acid requirements resembling those of man. Another principal inference from this work is that very complex compounds can arise in a very simple way. The proteinoids are, in our view, as complex as the proteins or perhaps a little more complex. In a nutritional context they can be viewed as synthetic generic protein.

In Fig. 2 are chromatograms of hydrolyzates of three separately synthesized proteinoids. These are by the automatic amino acid analyzer, using the analysis of Spackman, Stein, and Moore (14). The areas under the peaks correspond approximately to the proportions of individual amino acids, except for proline. Only the similarities and differences in pattern need be noted. The differences in each case are in the phenylalanine content. The central analysis is from a 2:2:1-proteinoid. The reaction mixture consisted of 40% aspartic acid, 40% glutamic acid, and 20% of an equimolar mixture of the sixteen other amino acids. This dry mixture has been heated to 170°C for six hours and then hydrolyzed under conditions that are used for hydrolyzing proteins and then analyzed in the usual fashion.

The analysis of the first of the hydrolyzed proteinoids is the same except that phenylalanine was omitted. The analysis shows, of course, that no phenylalanine is in the polymer, but all of the other amino acids are present in essentially the same proportions in the two syntheses.

This pair of patterns calls to mind the kind of situation that Abderhalden, Mendel, and others sought in proteins in nature in order to learn about the contributions of individual amino acids to protein nutrition. The comparison offers the possibility of feeding amino acids in peptide bound polymers with





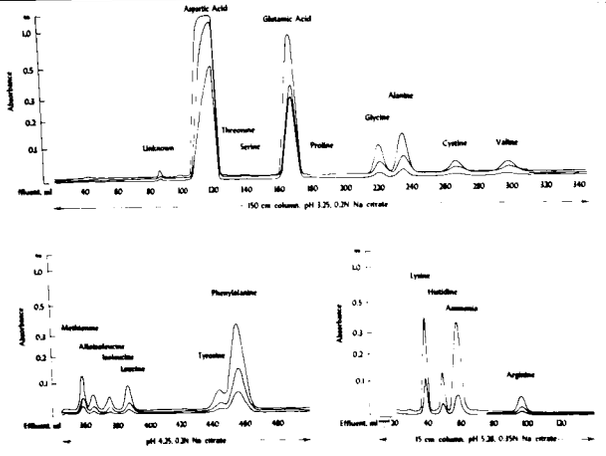


Fig. 2. Chromatograms of hydrolyzates of proteinoids.

a) 2:2:1-Proteinoid reaction mixture lacked phenylalanine.

b) Standard 2:2:1-proteinoid.

c) Same as b) with 3 1/2 times as much phenylalanine in reaction mixture.

systematic omission of individual amino acids. Information would thus be gained from bound amino acids rather than from free amino acids. Some of the defects of studies employing free amino acids have been pointed out by W. C. Rose (15) and reiterated by Dr. Hegsted at this meeting.

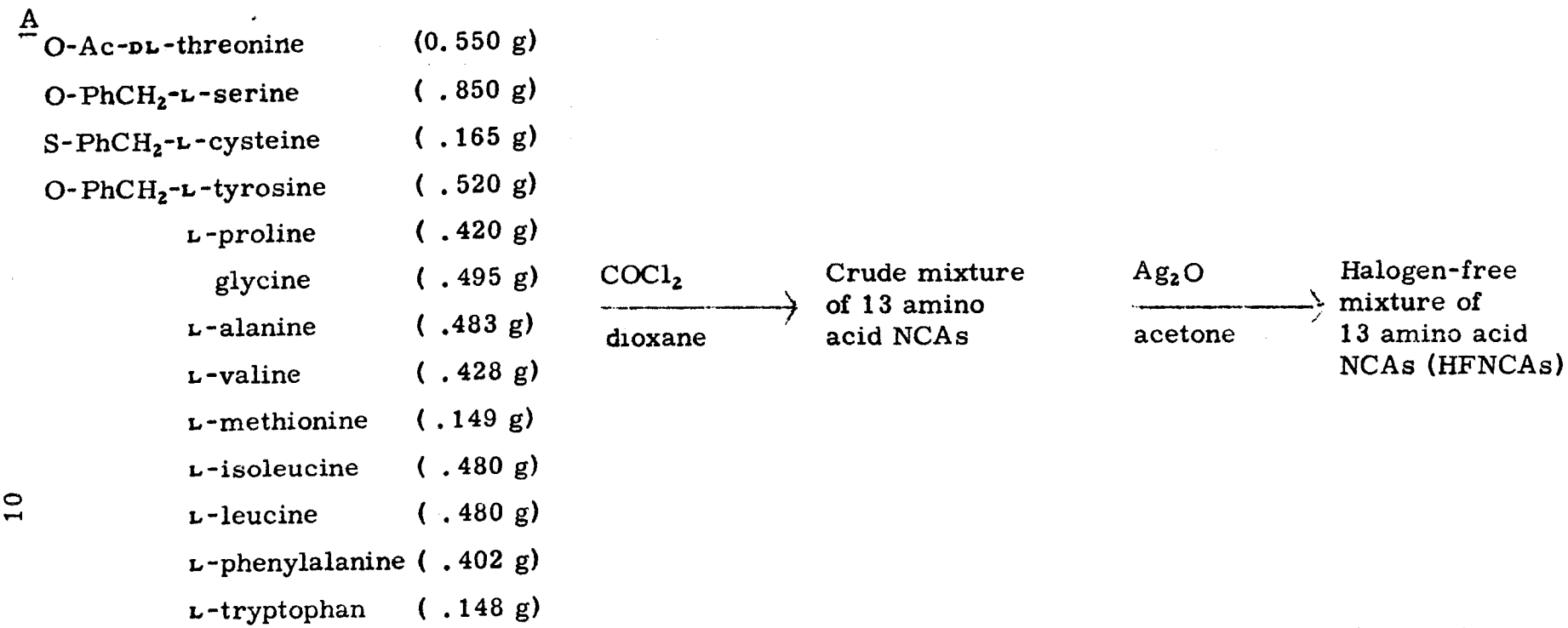
The third chromatogram shows the analysis of the hydrolyzate of a 2:2:1-proteinoid which was prepared under the same circumstances as the standard proteinoid except that three and one-half times as much phenylalanine was used in the reaction. One may see the larger proportion of phenylalanine in the proteinoid. Many similar studies demonstrate that the proportion of any amino acid in the thermal polymer is regularly related to the proportion of that amino acid in the reaction mixture. Proportions are, therefore, to a considerable degree controllable. Comparisons of the proportions of other amino acids show that the syntheses are highly reproducible. This kind of result has come as a surprise to many, although not all, chemists. This surprise may be understood on the basis that possibly heating has been erroneously regarded as invariably brutal treatment for amino acids and, secondly, because no precedent existed for simultaneous chemical polymerization of as many as eighteen monomers.

Much evidence has accumulated that the amino acids regulate their own sequences (3) as well as their composition, but this result is outside the area of our immediate concern.

Fig. 3 indicates another mode of producing proteinoids through the Leuchs anhydrides of the amino acids. This kind of synthesis is far from simple. It requires blocking of the reactive side chains of nine of the amino acids and then removal of the blocking groups, following polymerization.

The amino acids are not substantially racemized in this synthesis, however, and the proportions are less subject to internal control than are the proportions in thermal proteinoids. This product can simulate exactly a natural protein in proportions of individual amino acids.

Fig. 3. Flow sheet for a synthesis of Leuchs proteinoids.



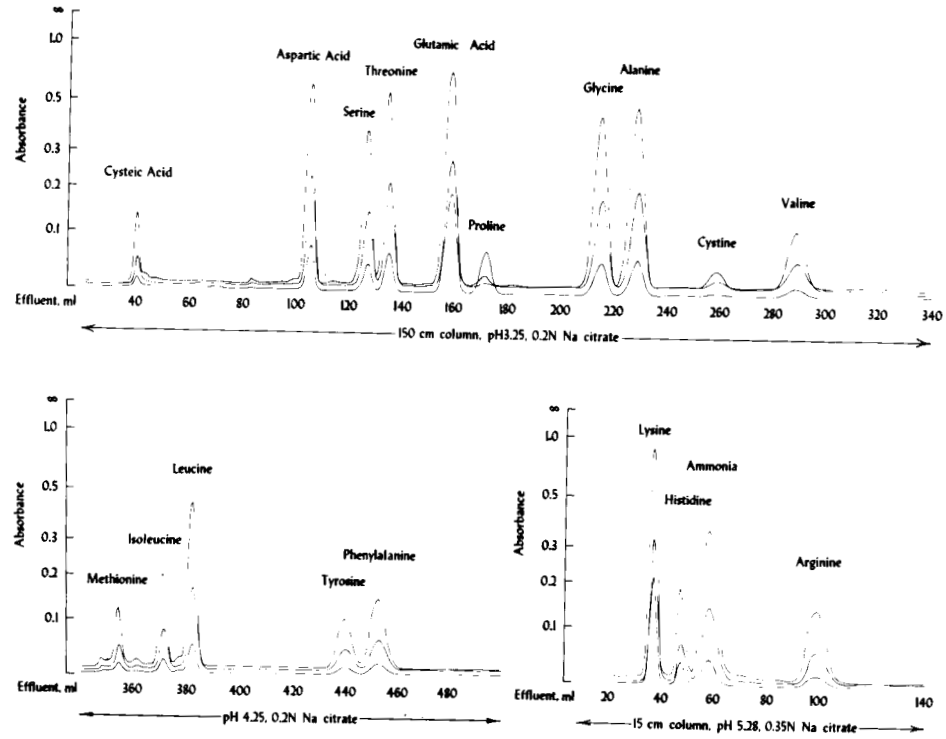
Fox, S. W., Hayakawa, T., and Windsor, C. R. (1963) Sept. meeting of the Amer. Chem. Soc., New York, Abstracts 7c.

In Fig. 4 are, again, a controlled pair of Leuchs proteinoids which show identical patterns except for histidine which has been omitted from one of the polymers.

In both this and the thermal type, proportions of otherwise nutritionally limiting amino acids, such as lysine, can be increased. For the objective of nutritional investigation, the thermal proteinoid and the Leuchs proteinoid each has its own features. They may be most valuable when used comparatively. Composition, as is indicated, can be controlled chemically and evaluated nutritionally. For the objective of space nutrition, the Leuchs proteinoid may be of particular investigative interest for missions requiring an optimally balanced nutritional polymer of amino acids. The thermal proteinoid should be more interesting for studies underlying chemical regeneration and studies aimed at prolonged missions.

Much more might be said about potential problems in the application of this relatively new knowledge to utilitarian objectives. Some of the problems are common to both chemical and biological regenerative systems. The pragmatic emphasis, I believe, is to recognize that many difficult problems can be visualized; that probably many problems are not yet defined, and to pursue the experimental avenues that are open. (The proteinoids, however, have acceptable taste, either raw or roasted, and they taste somewhat like grilled fish.)

To borrow a term from Dr. Bisplinghoff, we can now visualize a chemical option for investigation.



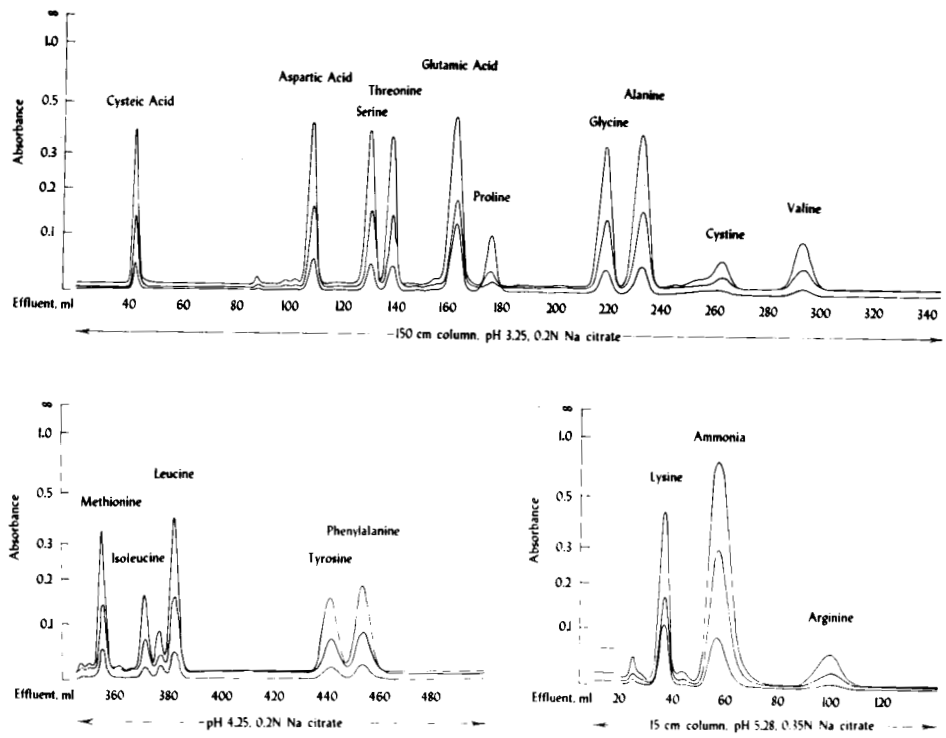


Fig. 4. Leuchs proteinoids:

a) all common amino acids,

b) lacking histidine.

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